Procedures for the Extraction and Purification of DNA

1 Scope

These procedures describe the isolation of deoxyribonucleic acid (DNA) from biological specimens recovered from evidentiary items for nuclear DNA typing.

2 Background

These procedures utilize two different lysis methods to affect the recovery of DNA that may be present in an evidentiary sample. The choice between methods is based on the serologically-determined presence of semen. A non-differential extraction method is utilized to isolate DNA from biological material that has tested negative for the presence of semen, or at a minimum, is not likely to be semen based on its commonly understood usage (e.g., bottles, cigarette butts, etc.). The differential extraction method is applied to those biological materials that have tested positive for the presence of semen and that are likely to contain other biological fluids as well (e.g., vaginal fluid, saliva, etc.).

These two methods provide the flexibility necessary to maximize nuclear DNA yields and fully exploit, when possible, the resistance of spermatozoa to lysis. This differential resistance to cellular disruption and lysis allows the collection of two separate nuclear DNA fractions: the female fraction (F), which is enriched with nuclear DNA of somatic cell origin, and the male fraction (M), which is enriched with DNA of sperm cell origin. In this way, the differential extraction method effectively allows certain, potentially mixed, specimens to be processed so that their resultant fractions are likely to display larger differences in the relative contributions of individual DNA donors. After lysis by either the non-differential or differential method, the specimens are purified by extraction using phenol/chloroform/isoamyl alcohol (PCI) and subsequent microdialysis through a microconcentrator.

3 Equipment/Materials/Reagents

Adjustable Pipettes (Rainin® Pipet-Lite®, Models L-2, L-10, L-20, L-100, L-200, L-1000, or equivalent)

Autoclave (Steris® Amsco® Eagle® Century SL Steam Sterilizer, or equivalent)

Barriered Pipette Tips (Rainin® Precision Pipette Tips, RT-L10F, RT-L200F, and RT-L1000F, or equivalent)

Basket Insert (Costar®, Spin-x® Insert, No Filter, 9301, or equivalent)

Biological Hood (AirClean™ 600 Workstation, Model AC600LFUV, or equivalent)

Biosafety Cabinet (LABCONCO Purifier Class II, Catalog # 36209-08 X, or equivalent)

Bleach cartridge refills (Market Labs, #VL102, or equivalent)

Centrifugal Filter Device (Amicon®, Inc., Microcon® SP YM-100, Catalog Number 42413, or equivalent)

Conical Tubes, 15 ml (Becton Dickinson, Falcon®, 352097, or equivalent)

Conical Tubes, 50 ml (Labcon, 3181-345-008, or equivalent)

Dithiothreitol (DTT) ($C_4H_{10}O_2S_2$, Sigma®, D-9779, molecular biology grade, or equivalent)

Disposable bench paper (Versi-dry, National Packaging Services, Part # 62070, or equivalent)

Ethyl Alcohol (Ethanol) [Pharmco, 200 proof (100%), Reorder Number 110002000PL05, or equivalent]

Ethylenediaminetetraacetic Acid Disodium Salt, Dihydrate ($C_{10}H_{14}N_2O_8Na_2 \cdot 2H_20$, Sigma®, E-5134, molecular biology grade, or equivalent)

Filter Purified Water [Barnstead Thermolyne NANOpure® Diamond™ Life Science (UV/UF) ultra pure water system, Series 1193, Model D11931, or equivalent]

Fume Hood (Fisher Hamilton, SAFEAIRE®, 54L0335, or equivalent)

General laboratory equipment and supplies (e.g., scissors, forceps, scalpel blades, rulers, tape, towels, etc.)

General purpose detergent/disinfecting solution (7930-00-926-5280, Lighthouse for the Blind, or equivalent)

Household Bleach

Hydrochloric Acid (Fisher Scientific, A-144, Reagent ACS grade, 36.5-38.0%)

Isopropyl Alcohol (70%) Wipes (Kendall Webcol® Alcohol Prep, Reorder Number 5110, or equivalent)

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Laboratory film (Pechiney Plastic Packaging, PM 996, or equivalent)

Mask (Kimberly Clark®, REF No. 62477, or equivalent)

Microcentrifuge (Hermle® MR-2, or equivalent)

Microcentrifuge Racks (Sarstedt, Number 93.837, or equivalent)

Microcentrifuge Tubes (Costar®, Catalog Number 3213, or equivalent)

N-Laurylsarcosine (Sarcosyl) ($C_{15}H_{28}NO_3Na$, Sigma®, L5125, or equivalent)

pH 4.00 Buffer (Ricca Chemical Company, NIST Traceable, Catalog Number 1501-16, or equivalent)

pH 7.00 Buffer Solution (Fisher Scientific, Certified, SB107-500, or equivalent)

pH 10.00 Buffer (Ricca Chemical Company, NIST Traceable, Catalog Number 1601-16, or equivalent)

pH Meter (Corning Incorporated, Pinnacle, 545 pH meter, or equivalent)

Phenol/Chloroform/Isoamyl Alcohol (PCI) (Invitrogen™ Life Technologies, Catalog Number15593-031, or equivalent)

Proteinase K (Amresco®, Biotechnology Grade, E195-25ML, or equivalent)

Refrigerator (4°C ± 3°C) / Freezer (-20°C ± 10°C)

Sodium Chloride (Sigma®, S-3014, molecular biology grade, or equivalent)

Sodium Dodecyl Sulfate (SDS) ($CH_3(CH_2)_{11}OSO_3Na$, molecular biology grade, Lauryl Sulfate, Sigma®, L-4390, or equivalent)

Sodium Hydroxide (Sigma®, S-5881, or equivalent)

Spray Bottle (Take 5®, Activate® Concentrate Dilution System, or equivalent)

Stationary Water Baths (Precision, Catalog Number 51221072, or equivalent)

Sterile filter (Nalgene® 0.45 µm, 115 ml, Catalog Number 121-0045, or equivalent)

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Transfer Pipettes (Samco Scientific Corporation, Cat. No. 232-15 or equivalent)

Trizma Base [Tris(hydroxmethyl)aminomethane, $C_4H_{11}NO_3$, Sigma®, T-8524, molecular biology grade, or equivalent]

UV Crosslinker (Spectronics Corporation, Spectrolinker®, Model XL-1000)

Vortex (Fisher Scientific, Touch Mixer, Model 231, or equivalent)

4 Standards and Controls

4.1 At least one extraction control (i.e., reagent blank) must be prepared and processed in parallel with each set of evidentiary specimens (i.e., non-differential extracted questioned specimens, differentially extracted specimens, known specimens, etc.) processed for potential DNA typing purposes.[1] The extraction control(s) contains all of the chemical solutions used in the analysis process except any DNA containing sample and is processed through the same extraction, quantification, amplification, and electrophoretic typing procedures as the evidentiary specimens. The raw data collected for each electrophoretic run of the extraction control(s) must be printed and included as part of the casework notes and electronic data. If more than one type of extraction procedure is employed (e.g., non-differential and differential extraction), at least one extraction control must be created and processed for each type of procedure.[2]

4.1.1 An extraction control monitors aspects of the analytical processes for the introduction of adventitious DNA on a potentially systemic[3] level or as a randomly occurring event. Although appropriate quality assurance practices are stringently applied and enforced, it is not unexpected that low-levels of adventitious DNA (i.e., levels that result in peak heights for allelic data that are below the Laboratory's reporting threshold) attributable to staff scientists directly involved in the analytical steps of such a highly sensitive technique may be detected. The optimum (and expected)

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[1] The extraction control tube(s) are created at the time of sample lysis. If sample collections are made by a Biologist and then transferred to unit storage to await further processing, the extraction control tube(s) should be prepared after the sample tube(s) is retrieved from storage and immediately prior to lysis set-up.

[2] If a nDNAU Examiner designates a questioned specimen (Q) as an "alternate known" prior to it being processed, this "alternate known" should be processed in parallel with its own extraction control (i.e., KB). If a questioned specimen (Q) is designated an "alternate known" after processing, the extraction blank (i.e., QB) run in parallel with it will serve as its required extraction control.

[3] As used here, "systemic" indicates those incidents of adventitious DNA in an extraction control that occur in a manner that suggests that the source of the introduced DNA was a material and/or reagent common to all of the samples processed as a case or batch.

outcome is that no DNA typing results be obtained from the extraction control(s). Sample quantity permitting,[4] any specimen(s) processed in parallel with an affected extraction control must be re-processed. If such re-processing is not possible, and the prescribed procedures have been followed, such resulting data may be interpreted in both a conservative and cautious manner.

4.1.2 If the DNA typing results observed in the extraction control: 1) are consistent with those from a staff scientist who conducted one or more of the procedure steps of the analysis, 2) display no allelic peaks above the Laboratory's allelic peak height reporting threshold, and 3) are not observed in any other sample or control processed in parallel with the affected extraction control, the event that introduced the adventitious DNA into this control is consistent with having occurred in a random (i.e., non-reproducible) fashion. The data for all of the samples processed in parallel with a control that displays randomly-introduced, adventitious DNA may be used for interpretive purposes and may be submitted for inclusion in CODIS. If the samples run in parallel with this control are the basis for the conclusions in a report (i.e., re-extraction, etc. was not possible and/or successful), the occurrence of randomly-introduced, adventitious DNA in an extraction control must be noted in the Laboratory report containing the results of the examinations.

4.1.3 If the DNA typing results observed in the extraction control: 1) are not consistent with those from a staff scientist who conducted one or more of the procedure steps of the analysis, 2) display one or more alleles that are above the Laboratory's peak height reporting threshold, and 3) are observed in one or more samples or controls processed in parallel with the affected extraction control, the event that introduced the adventitious DNA into this control is consistent with having occurred in a systemic (i.e., reproducible) fashion. The observation of adventitious DNA on a potentially systemic level demonstrates a failure in the analytical process. The data for all of the samples processed in parallel with a control that displays systemically-introduced, adventitious DNA may not be used for interpretive purposes and may not be submitted for inclusion in CODIS. The occurrence of systemically-introduced, adventitious DNA in an extraction control must be noted in the Laboratory report.

4.2 Non-Differential Extraction

4.2.1 The extraction control created as a part of the non-differential extraction procedure conducted on questioned (Q) specimen(s) of a case or batch is termed the QB.

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[4] As used here, "sample quantity permitting" designates those specimen's for which sufficient material (i.e., stain, friction material and/or isolated DNA) remains to conduct additional DNA examinations. It is noted that should the performance of any addition examination(s) result in the total consumption of a sample (i.e., no original material or isolated DNA would remain), an Examiner, or their designee, should contact the contributing agency or other responsible office (i.e., Office of the United States Attorney, etc.) concerning this necessity. Based on this communication, a mutually acceptable strategy should be developed concerning the future testing of such a sample(s).

4.2.2 The extraction control created as a part of the non-differential extraction procedure conducted on known (K) specimen(s) of a case or batch is termed the KB.

4.3 Differential Extraction

4.3.1 The extraction control created as a part of the differential extraction procedure is termed the DB.

4.3.2 The extraction control created in parallel with the isolation of the female fraction is termed the FB or DB(F).

4.3.3 The extraction control created in parallel with the isolation of the male fraction is termed the MB or DB(M).

4.4 The last sample to be processed during any step of the DNA isolation procedure must be the appropriate extraction control. This convention must be continued throughout the STR typing process, except for the handling of the positive and negative amplification controls which must be processed last.

5 Calibration

Not applicable.

6 Sampling

Not applicable.

7 Procedures

7.1 General Precautions

7.1.1 Laboratory Safeguards

7.1.1.1 Equipment

7.1.1.1.1 Only pipettes dedicated to pre-PCR amplification activities must be used when performing serological examinations, DNA isolation, and DNA quantification. Separate dedicated pipettes must be used when setting up PCR amplification reactions. Another dedicated set of pipettes must be used when handling specimens that potentially contain amplified DNA.

7.1.1.1.2 Pipettes dedicated to DNA isolation, DNA quantification, and PCR amplification, must be irradiated using the interior UV light of the biological hood for at least 5 minutes each workday before use. Also, they must be thoroughly decontaminated with a bleach solution then disinfected/rinsed with 70% isopropyl alcohol[5] each workday before use, as they become visually soiled and after their final use on a given workday. Pipettes dedicated to amplified DNA must be cleaned weekly[6] and as they become visually soiled.

7.1.1.1.3 Sterile disposable pipette tips or transfer pipettes must be used when handling liquid reagents or samples. Always use a new pipette tip when removing extract from a sample tube or when introducing reagent into a tube containing extract and discard the tip after usage.

7.1.1.1.4 To minimize the potential for pipetting inaccuracies, use the pipette with a range larger than and closest to the target volume. The pipette should be set to the desired volume by initially dialing into the range of volumes larger than the target volume and then dialing back to the desired volume.

7.1.1.1.5 New or clean forceps, scalpel blades, scissors, etc. must be used on a single sample and discarded, or they must be decontaminated with a bleach solution followed by 70% isopropyl alcohol between each use.

7.1.1.1.6 Before opening, quick-spin sample tube(s) in a microcentrifuge (approximately 2 seconds) to return all liquid to the bottom of the tube.

7.1.1.1.7 Sample tubes must remain closed unless being processed. Only one sample or reagent tube must be open at a time during the processing of the individual specimens of a case or batch through a common procedural step.

7.1.1.1.8 Biological hoods must be irradiated using their interior ultraviolet (UV) light for at least 5 minutes each workday before use.

7.1.1.1.9 For equipment which requires a calibration or performance check prior to use (e.g., pH meter, refrigerator, etc.), the results must be documented and maintained either electronically or as a hardcopy.

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[5] Bleach solution decontaminates the instrument or surface by removing any residual DNA or chemically rendering it unsuitable for analysis. The 70% isopropyl alcohol is a bactericide and removes any residual bleach that may adversely affect any biological material being processed with that instrument.

[6] Because of its corrosive nature, even as a dilute solution, 10% bleach is not used within laboratory space containing CE instrumentation (i.e., post-amplification space).

7.1.1.2 Personal Protective Equipment

7.1.1.2.1 Disposable gloves must be used at all times during the examination process to prevent the potential indirect transfer of biological material from one evidentiary item to another.[7] At a minimum, gloves must be changed if they become visibly soiled, torn, and before moving between dissimilar evidence items (i.e., between a vaginal swab and an oral swab, between a shirt and pants, etc.).

7.1.1.2.2 To prevent indirect transfer of biological material to telephones, computer keyboards, etc., used disposable gloves must be removed prior to handling such laboratory appliances. Double gloves may be worn to facilitate the removing and donning of outer gloves during those examination procedures in which notes are taken contemporaneously.

7.1.1.2.3 Laboratory coat, face mask, and gloves must be removed and hands washed prior to leaving the laboratory area.

7.1.1.2.4 Masks, gloves, bench paper, tubes, etc. that are visibly soiled with biological material (i.e., blood, semen, etc.) must be placed into biological waste containers for disposal. Disposable items that do not show any visible biological staining should be discarded into regular waste containers.

7.1.1.2.5 A dedicated laboratory coat must be worn during all pre-PCR amplification and PCR amplification processes. A separate, dedicated laboratory coat must be worn when handling specimens that potentially contain amplified DNA. Laboratory coats should be laundered upon becoming visibly soiled. All laboratory coats must be stored in their respective dedicated spaces when not in-use and must not be worn outside of designated laboratory space.[8]

7.1.1.2.6 Disposable face masks must be used at all times when handling evidentiary items or secondary evidence samples (i.e., cuttings and/or DNA sample tubes) to minimize the potential for introduction of biological material by Laboratory personnel.[9] At a minimum, face masks must be changed if they become visibly soiled or torn. Face masks must also be worn when preparing all reagents.

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[7] When handling evidence items of potential latent fingerprint value, cloth gloves should also be worn under the disposable gloves during processing.

[8] Soiled laboratory coats are collected (generally, weekly) and taken to the specified area.

[9] Face masks do not need to be worn during procedural steps making use of DNA aliquots that do not give rise to secondary evidence (e.g., CE sample preparation). Additionally, smear slides may be examined without a face mask provided there is a barrier between the biologist and the stage of the microscope.

7.1.1.3 Quality Assurance Safeguards

7.1.1.3.1 All work surfaces must be decontaminated with a 10% bleach solution each workday before use, as they become visually soiled, and after their final use on a given workday. All work surfaces within the post-amplification laboratory must be cleaned weekly with detergent and water.

7.1.1.3.2 Disposable paper (e.g., bench paper, weigh paper, tissue paper, etc.) must be used in processing evidence items to ensure a clean working surface and to prevent the deposition of biological material on permanent work surfaces.[10] Disposable paper must be changed as it becomes visibly soiled or, at a minimum, after the completion of the examination of an individual item of evidence and before the examination of another item is begun. Evidence items that are packaged together (e.g., vaginal swabs, clothing items, etc.) may be processed on the same disposable paper, provided that the paper does not display visible soiling.

7.1.1.3.3 Only one case will be examined at a time and only one Q specimen will be opened and collected for processing at any one time. The portion of the stain identified for analysis will be removed/collected from the Q specimen, placed into the corresponding labeled tube or envelope, and the Q specimen returned to the evidence packaging. This process is sequentially repeated for each Q specimen within the case.

7.1.1.3.4 All evidence items under active examination must be maintained as far as possible from other items of evidence under examination by any other individual(s) working within a common laboratory space.

7.1.1.3.5 Reagents must be stored separately from evidentiary samples. Separate storage in this context means, at a minimum, placing these reagents on a different shelf of the same storage used for evidentiary material. Reagents should be placed above evidentiary material in storage areas.

7.1.1.3.6 Controls samples must be tested in the manner prescribed in Section 4. The batch number and test results for all control samples must be documented in the casework notes.

7.1.1.3.7 Individual items from multiple Laboratory numbers may be referenced by use of abbreviated Laboratory numbers, such as the last four digits of the Laboratory number coupled with the specific item identifier (e.g. 1000 Q1, 1001 Q50, etc.). In the event a Biologist is assigned two cases for which the last four digits of the Laboratory numbers are identical, the two cases should be processed separately. With the prior approval of a nDNAU Examiner, cases that share their last four digits can be processed together (e.g., priority cases with short deadlines) using

[10] Used disposable paper that displays no visible contamination with biological material will be disposed of in regular laboratory trash.

expanded identifiers that uniquely distinguish each sample from the other evidence samples undergoing concurrent analysis. These identifiers should generally be based on the use of additional digits of the Laboratory numbers under concurrent examination.

7.1.1.3.8 All sample containing tubes that do not display a visible difference after the completion of a procedural step (i.e., color change, volume change, cutting introduction, etc.) must be physically moved or marked in a manner that distinguishes them from those on which that step has yet to be completed. This requirement will help to prevent the misloading or double-loading of samples during any procedural step that does not result in an evident physical change to a handled sample.

7.1.1.3.9 Notes taken during the examination of an evidence item (i.e., description, test result, etc.) must be taken immediately after the procedure (i.e., blood examination, semen examination, etc.) is conducted on that item. Such notes must be recorded in their final form (i.e., entered electronically into the casework worksheets) prior to beginning the examination of another evidence item of that case or batch. Multiple swabs from the same collection site and packaged together (i.e., vaginal swabs, oral swabs, etc.) may be processed together before being individually described within the final notes of examinations.

7.1.1.3.10 At the completion of testing, any remaining sample and extracted DNA must be packaged, labeled, and returned to the submitting agency in accordance with the *FBI Laboratory Quality Assurance Manual* and the *Nuclear DNA Unit Quality Assurance Manual*.

7.1.1.3.11 After all evidence has been returned and the final report issued, all remaining analytical materials generated as a part of the examinations conducted in nDNAU should be discarded (e.g., PSA extract tubes, etc.). Such material is not secondary evidence and is not returned with the items of evidence to the submitting agency.

7.1.2 Procedure Specific Safeguards

Not applicable.

7.2 Quality Control Procedures

7.2.1 All DNA isolation procedures[11] must be performed in dedicated laboratory space to maintain their separation from all sources of amplified DNA product.[12]

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[11] As used here, the DNA isolation procedures begin with the addition of extraction buffer to the sample recovered for DNA typing.

[12] Amplified PCR product must be stored in a separate room from those where DNA isolation may occur.

7.2.2 All DNA isolation procedures must be conducted within self-contained hoods unless otherwise indicated.[13]

7.2.3 All DNA isolation procedures must be performed using pipettes dedicated to pre-PCR amplification reaction set-up activities.

7.2.4 To maintain an individual sample's separation in time, each step of the nuclear DNA isolation procedures must be completed on a sample, and that sample tube closed, before that step is performed on any other sample within a case or batch.

7.2.4.1 The DNA isolation reagents must be dispensed into all of the tubes containing Q specimens individually with only one Q specimen tube being open at a time. All Q specimen tubes may then be incubated together.[14]

7.2.4.2 The DNA isolation reagents must be dispensed into all of the tubes containing K reference specimens individually with only one K specimen tube being open at a time. All K specimen tubes may then be incubated together.

7.2.4.3 Following the sequential addition of lysis reagents to the isolation tubes (i.e., Q specimens before K specimens), tubes containing Q specimens may be incubated together with tubes containing K specimens within a common water bath.

7.2.4.4 Following completion of the extraction and purification processes for the Q specimens; those steps are then performed on the K reference samples.

7.2.4.5 The Q specimens and K reference samples will be grouped together according to Lab numbers through the quantification, amplification and capillary electrophoresis and processed as individual samples.

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[13] As an example, all nuclear DNA isolation steps in which phenol/chloroform/isoamyl alcohol (PCI) reagent is used must be performed in a fume hood to minimize exposure.

[14] Evidence specimens assigned a known (K) specimen identifier for comparative examinations within other forensic disciples (e.g., guns submitted for firearm examinations) can be processed together with question (Q) specimens for the purposes of nuclear DNA analysis examination.

7.3 Reagent Preparation[15, 16, 17]

Graduated cylinders and/or pipettes closest in capacity to the volume of liquid being measured must be used. Unless otherwise prescribed, reagents may be used for up to a calendar year after their date of preparation. Any reagent in which microbial growth is observed must be discarded.

7.3.1 Autoclaved Filter Purified Water

Autoclave filter purified water under standard conditions. Store at 4°C ± 3°C. In-use aliquots of autoclaved filter purified water may be stored at room temperature for up to one month.

7.3.2 1 M Dithiothreitol (DTT), 10 ml

Dissolve 1.54 g of DTT in 10 ml of filter purified water in a sterile, disposable plastic 15 ml conical tube. Do **not** autoclave. Aliquot the solution and store the aliquots at -20°C ± 10°C. Discard any unused portion of a thawed aliquot.

7.3.3 Ethylenediaminetetraacetic acid (EDTA), 0.5 M, pH 8.0, 2 L

Slowly add 372.2 g of EDTA to 1600 ml filter purified water. Stir vigorously on a magnetic stirrer. Adjust to pH 8.0 by adding 10 N NaOH using a calibrated pH meter. EDTA will not go into solution until pH is adjusted. Adjust final volume to 2 liters with filter purified water. Autoclave. Store at room temperature.

7.3.4 20% (w/v) N-Laurylsarcosine (Sarkosyl), 100 ml

Add 20 g of Sarkosyl to 70 ml filter purified water and stir until dissolved. Bring to a final volume of 100 ml with filter purified water and sterilize by passage through a 0.45 µm filter. Aliquot the solution and store the aliquots at 4°C ± 3°C. In-use aliquots of 20% Sarkosyl may be stored at room temperature for up to one month.

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[15] Except where noted, all reagents are stored in glass containers (i.e., bottles, etc.).

[16] Refer to nDNAU 701 *Procedures for Use of the Steris Autoclave* for all reagents that require autoclaving.

[17] Upon expiration of a reagent batch, any daughter solution(s) made with this expired parent reagent must also be deemed expired. All reagents containing components with expiration dates that precede the prescribed expiration period of the solution being prepared must have their expiration date adjusted to accommodate the expiration date of the oldest component used in making the reagent. For example, if solution X, which has a prescribed expiration of 1 year after preparation, contains component A that expires 6 months from the preparation date of solution X and component B expires in 3 months from that date, solution X must be assigned an expiration date of 3 months.

7.3.5 20% (w/v) Sodium Dodecyl Sulfate (SDS), 2 L

Slowly dissolve 400 g of SDS in 1600 ml filter purified water. To aid dissolution, solution may be gently heated (generally a setting of 2 to 3). Adjust volume to 2 L with filter purified water. Store at room temperature.

7.3.6 5M Sodium Chloride (NaCl), 1 L

Dissolve 292.2 g of NaCl in approximately 700 ml filter purified water. Adjust volume to 1 L with filter purified water. Autoclave. Store at room temperature.

7.3.7 10 M Sodium Hydroxide (NaOH), 1 L

Dissolve 400 g NaOH in approximately 700 ml filter purified water. Because this mixing is exothermic in nature, add the NaOH pellets slowly to avoid excessive generation of heat. Adjust volume to 1 L with filter purified water. Store at room temperature.

7.3.8 Sperm Wash Buffer

10 mM TRIS-HCl / 10 mM EDTA / 50 mM NaCl / 2% SDS, pH 8.0, 1 L

Add 10 ml 1 M Tris-HCl, pH 8.0; 20 ml 0.5 M EDTA (pH 8.0); 10 ml 5 M NaCl; and 100 ml 20% SDS to 860 ml filter purified water. Check pH with a calibrated pH meter and adjust to pH 8.0 with concentrated HCl or 10N NaOH as necessary. Adjust volume to 1 L with filter purified water. Autoclave. Store at room temperature.

7.3.9 Stain Extraction Buffer (SEB)

10 mM TRIS-HCl / 100 mM NaCl / 10 mM EDTA / 2% SDS, 1 L

Dissolve 5.84 g NaCl in 500 ml filter purified water with stirring. To this solution add 10 ml 1 M Tris-HCl, 20 ml 0.5 M EDTA, and 100 ml 20% SDS. Titrate to pH 8.0 with HCl using a calibrated pH meter. Bring to a final volume of 1 L with filter purified water. Store at room temperature.

7.3.10 Stain Extraction Buffer (SEB) with Dithiothreitol (DTT)

10 mM TRIS-HCl / 100 mM NaCl / 10 mM EDTA / 2% SDS / 39 mM DTT

Before use, SEB must be fortified with DTT at a concentration of 6.02 mg/mL. Add 301 mg of DTT to 50 mL of SEB and stir until dissolved. Store at room temperature. DTT fortified SEB may be used for up to 1 month.

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